very resistant to squill poisoning but very high feeding levels may be toxic. These rabbits underwent the same type of rolling convulsion as do rats preliminary to recovery or death from red squill poisoning. No vomiting was observed.

Toxicity to Chickens.—Chickens were also fed red squill extract in like manner. The feeding levels used ranged from 450 mg./Kg. to 4500 mg./Kg.

This experiment indicates that red squill extracts are non-toxic when fed to chickens, even in very large amounts.

Red squill powder was fed to chickens on the following levels: 2400 mg./Kg., 3200 mg./Kg., 4000 mg./Kg. and 4800 mg./Kg. No discomfort was observed following the squill powder feeding. This corroborates the findings of other investigators relative to the innocuous character of red squill powder when fed to chickens.

DISCUSSION

Rats, guinea pigs, rabbits and chickens are unable to vomit. However, rats and guinea pigs are susceptible to red squill poisoning while rabbits and chickens are not. It therefore seems that poisoning from red squill is due to a species susceptibility rather than any ability or inability to vomit. It should be noted, of course, that vomiting may protect susceptible animals from red squill poisoning by ridding the animal of the poison.

SUMMARY

1. Chickens and rabbits do not seem to be affected by levels of red squill extract and red squill powder which are definitely toxic to rats. However, rabbits may be susceptible to very high levels of red squill dosage.

2. Guinea pigs are less resistant to poisoning by red squill extract and powder than are rats.

3. It is believed that red squill poisoning is dependent on species susceptibility rather than on ability or inability to vomit.

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On the Preparation of an Extract Having "Marihuana-Like" Activity from the Fruits of Cannabis Sativa

By John R. Matchett and S. Loewe†*

Despite the considerable research carried on in recent years on the separation and characterization of the physiologically active principle or principles of the resin of *Cannabis sativa*, little has been reported concerning the fruits. Munch (1) prepared two physiologically active extracts and found one to be stimulating to mice and the other depressant. Reference to action similar to that of extracts prepared from the vegetative portions of the plant was not made.

Bouquet (2) has examined the fruits and concludes that they themselves are devoid of physiological activity. Any effects observed pursuant to their use he feels must be due to the presence of hulls which do contain resin.

This report records the unequivocal demonstration of the presence in cannabis fruits of a principle or principles having, in the dog, an action similar to that produced by the familiar cannabis resin. The substance or substances are to be found in the non-saponifiable portion of the solvent-extracted oil which is a major constituent of the fruits.

EXPERIMENTAL

The preparation of the active extract was carried out as follows:

Fruits of *Cannabis sativa* (675 Gm.), free from admixed vegetative material, were crushed and extracted to exhaustion with chloroform. Most of the solvent was evaporated at atmospheric pressure and the last traces removed *in vacuo*. The oily

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residue weighed 234 Gm. (33%). This material was dissolved in 750 cc. of 1.25*N* alcoholic potassium hydroxide and the solution was boiled under reflux until saponification was complete. The resulting soap solution was diluted with an equal volume of water and extracted continuously with pentane (Skellysolve *A*) to exhaustion. The pentane solution was washed with water until free from soap, then evaporated on the steam bath. The residue consisted of 4 Gm. (0.6%) of resinous oil in which some crystals were discernible.

The residue was extracted with 35 cc. of alcohol which dissolved most of the material except the crystals and a little resinous matter. The crystals were dissolved in 25 cc. of acetone and the resinous residue in 5 cc. of chloroform. The three solutions were referred to as samples number 1, 2 and 3, respectively.

The crushed fruits remaining in the percolator were extracted to exhaustion with alcohol and the extract concentrated to 250 cc. *in vacuo*. This sample was designated number 4.

The capacity of the preparations to exert a "marihuana-like" action was tested in the dog. When symptoms of motor ataxia (the characteristic effect of cannabis preparations in the dog) were observed in test experiments, the potency of the preparation was evaluated by the procedure of "bioassay by approximation" recently described (3, 4).

The preparations were administered by intravenous injection in a concentrated ethanol or acetone solution, and the intensity of action was recorded in grades (I to VI; according to the scale used by Walton and collaborators (5)).

Sample 2, the ethanol-insoluble, but acetone-soluble fraction from the pentane residue, was found ineffective. This agrees with the experience that in the case of highly potent cannabis tops, under analogous procedures, the active principle is taken up by ethanol extraction and no appreciable amount is left for a subsequent acetone extraction. Accordingly, no activity could be expected in Sample 3 which could not be tested due to its insolubility in any appropriate solvent. Sample 4 was also found ineffective in accordance with the fact that another extract prepared by direct ethanol extraction of defatted seeds was also found devoid of any specific action in the highest dosage which could be administered without non-specific toxic effects.

The main fraction, however, Sample 1, produced varying degrees of ataxia in seven experiments, as shown in Table I. All these experiments were carried out in dogs which had been satisfactorily calibrated with a standard of reference, a highly potent redistillate oil preparation from cannabis tops, before and after the test experiment, and, therefore, the principle of "bioassay by approximation" was applicable to these experiments. Since the application of this assay method to cannabis preparations has hitherto not yet been published *in extenso*, it might be appropriate to exemplify its use in the case of these experiments. This is done in the second part of Table I. This part of the Table enumerates the figures of Maximum and Minimum Potency (3, 4) which were obtained as the ratios between a dose of the standard preparation employed in calibrating the same test dog and the dose of the test preparation, Sample 1. Each ratio obtained under use of a calibration dose which was more effective than the test dose indicates a "Maximum Potency," each ratio with a calibration dose of lower effectiveness than the test dose indicates a "Minimum Potency." These values, grouped in arithmetical progression, as in Table I, show at once the range of potency outlined by the group of experiments; and therefrom a median value of potency can be calculated, as at the bottom of the approximation.

Table I.—Bioassay by Approximation of Non-Saponifiable, Ethanol-Soluble Portion (Sample 1) of Hemp Seeds

Experiments

Experiment No.	Dog No.	Dose in Equivalents of Gm. Seed per Kg.	Intensity of Action
383	9	6.4	IV–V
593	47	5.7	II–III
594	49	5.2	II–III
384	40	4.8	I–II
592	9	3.8	II–III
498	49	3.5	II
554	61	3.2	I
488	49	2.5	II
380	43	1.9	I–II
543	49	0.9	I

Calculation

Potency of Experiment No.	Values in Sequence of Approximation	
	Minimum Potency	Maximum Potency
384	0.040	...
383	0.060	...
594	0.067	...
592	0.100	...
383	0.105	...
380	0.116	...
594	...	0.133
592	...	0.133
593	...	0.137
554	...	0.145
592	...	0.159
380	0.211	...
498	...	0.213
384	...	0.220
592	...	0.228
383	0.232	...
594	...	0.267
380	...	0.291
488	...	0.296
543	...	0.371
383	...	0.416
380	...	0.422
592	...	0.444

Potency = 0.187×10^{-3}.
Range of Deviation: ±33%.

For the sake of convenience, the potency values were multiplied by 10^3 before they were entered into the two last columns of the second part of Table I. The potency value determined in this assay refers to the starting material of Sample 1, *i. e.*, to clean fruits of *Cannabis sativa*, as compared with a potent

redistillate marihuana oil. Considering, accordingly, only the amounts of active principle which were recovered from hemp seeds after saponification of its chloroform extractives, hemp seeds would appear to possess about $^1/_{5000}$ the potency of the marihuana oil employed as standard of reference. This, according to our bio-assays of a great variety of cannabis tops, would signify that hemp seeds were about $^1/_{10}$ to $^1/_{30}$ as potent as the carefully ground and screened parts of dried cultivated tops from various (*e. g.*, Roumanian, Manchurian, Italian) varieties of *Cannabis sativa*, or $^1/_{30}$ to $^1/_{100}$ as potent as herbs from the illegitimate marihuana trade.

CONCLUSIONS

Qualitatively, these experiments give evidence of the presence of an active principle in hemp seeds which is similar in its pharmacological action to that of marihuana oil. Quantitatively, the value of potency found in the present preparation may help to explain why, in quite a number of attempts, we were unable to elicit "marihuana-like" action with less intricately prepared extracts from either total, or crushed, or defatted cannabis fruits, but this potency figure cannot be considered as conclusive with respect to the amount of active principle present in the starting material of our preparation. It is hardly more than a minimum value, and it is open for further investigation to determine how much of the active principle originally contained in the fruits may be lost in preparing this non-saponifiable fraction either by reasons of chemical destruction or due to insufficiencies of the procedures of fractionation.

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"Nothing would be done at all if a man waited till he could do it so well that no one could find fault with it."—John Henry Newman.

Alkyl Nitrites. VII

Synthesis of Some Organic Nitrites and Nitrates*

By Sylvan E. Forman, C. Jelleff Carr and John C. Krantz, Jr.

In the course of certain studies on the pharmacology of nitrites (1, 2, 3) and nitrates (4, 5), it was necessary to synthesize a number of organic compounds. We are reporting in this paper the data accumulated on the chemistry of these substances.

The nitrites were prepared from the corresponding alcohols and nitrous acid in the same manner as described for the preparation of 2-ethyl-*n*-hexyl-1-nitrite. Myristyl, cetyl and *n*-octadecyl alcohols were dissolved in diethyl ether before they were treated with nitrous acid. Nitrite nitrogen was determined in these compounds by the nitrometer method described for the assay of amyl nitrite in the U. S. Pharmacopœia X, page 49. In the analysis of myristyl, cetyl and octadecyl nitrites, the aqueous potassium iodide solution had to be replaced with potassium iodide solutions in 75 per cent ethanol, because of the greater insolubility of these nitrites in water.

Propyl, butyl and heptyl glycollates were prepared by fractionating mixtures of glycollic acid, benzene and a little sulfuric acid with an excess of the corresponding alcohol as in the description of the preparation of the propyl ester.

The nitrates were prepared in the manner described for the preparation of isoamyl lactate nitrate. In some cases it was necessary to pour the nitrating mixtures into ice water to obtain the product. These compounds were analyzed by the well-known modification of the Kjeldahl method which employs salicylic acid and sodium thiosulfate to reduce the nitrates.

In addition to the compounds listed in the experimental portion, the following were also prepared but the analyses indicated that they were not obtained pure: *n*-nonyl-

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